ON THE PHYSIOLOGY OF THE RECTAL GILLS IN THE LARVÆ OF ANISOPTERID DRAGONFLIES.

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(Plate xlvii.)

The physiology of respiration is one of the most difficult problems still awaiting a solution in the biology of the Insecta. Most insects breathe by means of *spiracles*, which are the apertures of a complicated system of air-tubes or *tracheæ* ramifying throughout the body, and ending internally in numerous excessively fine tubes or *capillaries*, which supply oxygen directly to all the cells concerned in the metabolism of the body-tissues. Such a system is known as an Open Tracheal System. There are, however, certain aquatic larvæ in which spiracles are either absent, or, if present, non-functional, except, perhaps, on very special occasions, such as when the larvæ leave the water just prior to metamorphosis. In these forms, the required oxygen is derived from the water either directly through the integument, or, more usually, by means of *tracheal gills*. To this latter group belong the larvæ of Mayflies, Stoneflies, and Dragonflies. This type of system is called a Closed Tracheal System.

It is not the purpose of this paper to discuss the various theories of respiration which have been put forward to meet the case of the Open Tracheal System. The question, whether expiration is performed through the spiracles, as well as inspiration, or whether the latter alone takes place there, does not enter into the problem which we have before us. For the fact, that the tracheal system of the Dragonfly Larva is *physiologically* a closed one, cannot be disputed, though this closed system shows clearly a secondary derivation from an original open system, resembling that of the imago. Spiracles are present both on the thorax and the abdomen. Those of the thorax, at any rate, can, under certain circumstances, be made to function as orifices of inspiration *at any time that the larva chooses to leave the water.* But we are concerned here only with the problem of *aquatic* respiration, as carried on, day in and day out, throughout the whole life of the growing larva. The admitted facts that *certain* Dragonfly larvæ do occasionally crawl out of the water and breathe air directly through their spiracles, and that probably *all* Dragonfly larvæ also use their spiracles in this way for a short time just prior to metamorphosis, do not affect the general question, and cannot be used as an argument against the conclusions to be arrived at.

I have chosen to restrict the problem in two directions. Firstly, I shall deal only with the Suborder Anisoptera, *i.e.*, those dragonflies whose larvæ breathe by means of *rectal* gills, and shall not consider the somewhat different problem presented by the Zygoptera, whose larvæ breathe by means of *caudal* gills. This is done because our knowledge of the morphology of these latter organs is still in a very backward state, and does not as yet offer a sufficiently sound basis on which to build any general physiological conclusions. The morphology of the rectal gills of Anisoptera, on the other hand, is by now fairly well known, so that we are able to approach the physiological problem with some hope of success. Secondly, I do not intend to offer an explanation of the working of the whole respiratory system, including a detailed discussion of the elimination of nitrogen and carbonic acid gas from the body. The problem before us is to find a satisfactory explanation of the method of obtaining the oxygen, necessary for the metabolism of the larva, from the water, and its distribution to all parts of the body.

It will not be necessary to explain in detail the differences between the various types of rectal gills. I have already dealt very fully with these in a previous paper(8), to which I would refer anyone desirous of studying the comparative morphology of these beautiful and interesting structures. It will be sufficient here to state that the different forms can be classified primarily into two main divisions, the Simplex System and the Duplex System. In the former, the rectal wall is thrown into six main longitudinal folds, forming continuous gills from end to end of the branchial basket; each of these *main-folds* is supported at intervals, to right and left alternately, by smaller *cross-folds*. In the Duplex System, the main-folds are functionless or suppressed, and the cross-folds become separate gills, arranged in six double-rows. Further, the gills of the Simplex System may be classed as either of the Undulate or Papillate Type, while those of the Duplex System may be either of the Implicate, Foliate or Lamellate Type. Whatever be the type of gill, it remains essentially a delicate eversion of the rectal wall, projecting far into the rectal cavity, and bathed on all sides by the water drawn into that cavity. I have contented myself, therefore, with figuring only the gill of the larva of *Hemicordulia tau* Selys (Plate xlvii.). This belongs to the Lamellate Duplex Type, and is, at the same time, both one of the most highly organised, and one of the most easily understood of all rectal gill-types.

On opening the branchial basket (*i.e.*, the anterior portion of the rectum) of this larva, we find a very beautiful and regular arrangement of gills within. There are six, longitudinal, double rows of lamellate gills (Fig.1) of a beautiful pale mauve tint. Each single row contains twenty or more of these separate lamellæ, in the form of semiovals inserted on a broad base (Fig. 2*b*), placed obliquely, and overlapping one another from before backwards. .Consecutive lamellæ are protected from rubbing against one another by the action of three small tubercles (Fig. 2, *ta*, *tp*), which also allow a sufficient space between them for a complete circulation of water around the gill.

On the outer anterior face of the base of each gill, there is a large rounded swelling, the basal pad(*bp*), very conspicuous, and of a semitransparent orange-colour. This pad is a turgid structure, apparently full of watery fluid. The boundaries of the cells forming it are obliterated, but their nuclei are large, and form centres from which very distinct fibrillar structures pass out to the borders of the pad. Beneath the pad, the space between the bases of the two walls of the gill-lamella is filled with a mass of *hypobranchial tissue* (*hb*), surrounding the region of the large efferent tracheæ of the gill. The nature of this tissue is not clearly understood, but it seems to be a derivative of the fat-body, and obviously lies in the hæmocœle. In fact, it practically blocks out the circulation of the blood from the gill, in which definite blood-spaces do not really exist, except around the larger tracheæ near the base. In the lamellæ itself, although the two walls of the gill can be slightly forced apart by the artificial injection of fluid (thus proving the continuity of the hæmocœle into this organ), yet no circulation of blood-corpuscles has ever been observed; and, so far as my own observations go, such circulation would be impossible, owing to the fact that the two walls of the gill are, in the natural state, practically in contact everywhere except at the base. In other forms, where tracheæ of considerable calibre penetrate some distance into the gill, blood-spaces are met with further in (*bs*) around these tracheæ. In the Papillate type, the blood-spaces follow the axial trachea of the papilla almost to its tip. But in no case is there any noticeable circulation of blood, and that fluid does not play any important part in the problem of oxygen-carrying.

The gill is covered everywhere by a very fine transparent *cuticle* (*cu*), representing the *intima* or chitinous lining of the undifferentiated rectal epithelium. Beneath the cuticle lies the gill-epithelium (*ep*). This is a thin layer of very flattened cells, all fused together into a syncytium in which the nuclei (nu_1) are clearly visible at somewhat irregular intervals, and placed rather far apart. The mauve colour of the lamellæ of *H. tau* is due to the presence of very fine granules of purplish pigment (*pg*) scattered in this syncytium.

The arrangement of the gill-tracheæ can be gathered from Fig.1. Almost the whole of the lamella proper carries only capillaries (*cap*). These are very numerous and excessively fine tubes, with no trace of a spiral thread. It is very important to note that each capillary forms a *complete loop*, passing up one side of the gill, crossing over beneath the rounded distal border of the lamella, and descending along the other side, finally joining up with other capillaries to enter a larger branch which unites, at last, with the branch from which the capillary took its rise. Under a high power, the capillaries can be seen to lie actually *within* the epithelial syncytium, *fairly close up to the cuticle* (Fig.5).

The capillaries are collected into bundles at the base of the gill, forming five or six, stouter, short tracheæ. These unite together to form the efferent trachea of the gill, termed a *secondary efferent* (*es*). They are surrounded by the hypobranchial tissue at the base of the gill. The secondary efferents from the gills on one side of a double-row unite in pairs with those from the gills of the other side, forming a single longitudinal set of *primary efferents*, which enter one of the main longitudinal trunks of the body.

We can now attack our problem directly. There are clearly eight separate elements which go to form a single gill, viz., (1) the cuticle, (2) the epithelial syncytium, (3) the pigment, (4) the basal pad, (5) the hypobranchial tissue, (6) the blood-plasma in the narrow blood-spaces, (7) the capillaries, and (8) the larger tracheæ and efferents. We have to determine which of these are the essential elements in respiration, and which are merely accessory thereto.

We must class as accessories all those structures which are not present in all types of rectal gill. For if, in certain cases, the gill respires satisfactorily without them, it is clear that they have a function only accessory to respiration in those gills in which they are present.

It is also clear that the part of the gill, which is concerned with the extraction of oxygen, is the part which projects into the water in the rectal cavity. Structures which are present only at the base of the gill will, in general, therefore, be only accessories.

Thus we arrive at the following list of accessory structures:—

(1). *The pigment.*—Although developed all over the lamella in *Hemicordulia*, yet, in most forms, the pigment is confined to the basal part of the gill. In many genera (e.g., *Austrogomphus*, *Petalura*, *Cordulegaster*, *Dendroœschna*, *Austrophlebia*, *Orthetrum*) *no pigment is developed in any part of the gill.* Hence we may safely conclude that the pigment is purely an accessory element, so far as respiration is concerned.

(2). *The basal pad.*—Not only is this developed at the base of the gill, but it is either absent or very feebly developed in gills of the Implicate Duplex Type, as well as in the gills of the majority of *young* larvæ. Hence we may not only class it as an accessory, but we may gather that its chief function is that of a support for the gill. For, in the implicate duplex type, the gills overlap one another in the form of a series of concave tiles with rounded ends, these ends being connected together so as to form a continuous wavy ridge. Thus the two rows of gills in one double-row support one another, and no basal pad is needed. The fact that the basal pad is absent or vestigial in this type of gill points, therefore, to its functioning in general as a support for the gill.

(3). *The hypobranchial tissue.*—This also is absent or feebly developed in young larvæ, and also in gills of the Implicate Duplex Type, as well as being developed, in other forms, only at the extreme base of the gill. Hence we may class it as an accessory. It is possible, however, that this tissue may play some part in the physiology of respiration, apart from that of oxygen-extraction. It may, for instance, help in the removal of nitrogen or carbonic acid gas.

(4). *The blood-plasma.*—We class this as an accessory, partly because there is no definite blood-circulation in the gill itself, and no clearly defined blood-spaces except at its base, and partly because the blood of insects (except in a few very special cases) appears to be entirely lacking in oxygen-carrying elements. We must, however, accord to the blood an important part in the removal of carbonic acid gas from the body, and perhaps also portion of the nitrogen.

Thus, out of eight elements of the gill-structure, four are seen to be accessories. We must next remove from the problem a fifth, viz., the larger tracheæ, since these clearly function only as *receivers of oxygen from the capillaries*, *i.e.*, efferents to the main tracheal system. Thus the problem is now reduced to the consideration of three elements, viz., the *cuticle*, the *epithelial syncytium* (minus the pigment), and the *tracheal capillaries*. How do these three combine for the extraction of oxygen from the water in the rectal cavity?

An examination of Figs.3, 5, will at once suggest the strong probability that the gas is obtained *by a simple process of diffusion through the slender wall of the gill into the underlying capillaries* The points in favour of this are: –

i. The thinness and delicacy of the cuticle.

ii. The alteration of the undifferentiated rectal epithelium (Fig.6*b*) into a thin syncytium, with elimination of cell-boundaries.

iii. The extreme fineness of the walls of the tracheal capillaries, and the complete absence of the spiral thread.

iv. The fact that the capillaries, instead of running in a definite blood-channel, are embedded in the protoplasm of the epithelial syncytium, thus becoming arranged as close to the external surface of the gill as is possible under the circumstances.

v. The fact that all the capillaries form *complete loops.* For, if oxygen were extracted at any given point, or by any given localised organ, we should expect the capillaries to *end* in that point or organ. The fact that they form complete loops shows, however, that the extraction must take place at all points along the circumference, and pass inwards down both arcs of the capillary, to its junctions with the larger efferents. In other words, there is no *circulation* of gas in the capillaries in a definite direction round the circumference, but simply a passage of gas from all points inwards towards the efferent tracheæ.

Some such solution as this had evidently been in the minds of most of the earlier students of the subject, until certain apparently insuperable difficulties were pointed out. On that account, the Diffusion Theory, as I propose to call it, became unpopular, in spite of Lowne's advocacy(**3**), until Ris again recently reviewed it—without, however, clearing away the initial difficulty of the problem As I intend, in this paper, to support the view advanced by Lowne and Ris, it will be as well if I make their position clear by quoting a translation of the latter's own words(**5**):—The question becomes more difficult in the case of the Closed Tracheal System, of which our larvæ furnish a classical example. What is here wanting is the *vis a tergo* of the inspiration-organ, and we are driven to this, to assume purely chemical or, as the case may be, diffusion-forces as the motive agent ("rein chemische, resp. Diffusionskräfte als das bewegende Agens anzunehmen"). Lowne postulates for this, and also further on, for the function of the Closed System, at least a temporary opening of the thoracic stigmata, which certainly are not wanting in our instance. The difficulty lies here especially in this, that such a function of these stigmata is only concluded ("nur erschlossen"), but never positively observed.

On this ground, Ris rejects Lowne's theory on this single last point, and declares the problem not yet solved It seems quite clear that Ris is right, for the following reasons. Supposing we grant Lowne's contention concerning the thoracic spiracles, what then? In the case of aquatic larvæ, of what avail would the opening of these spiracles be, seeing that only water, and not air, could be drawn in through them? Or, if it be urged that, at any rate, the young larva might climb out of the water for a short time, and thus fill its tracheal system with air through its temporarily opened spiracles, the answer is very definite, viz., that no such action by the larva takes place, and that *the first filling of the tracheæ with air has been observed more than once to take place beneath the water.*

Thus the opposition to the Diffusion-Theory comes simply to this, that the main principle of the Theory is not even attacked, but is admitted to be exceedingly probable, were it not for the supposed insuperable difficulty attending the *starting of the process* in the newly-hatched larva. It is well known that the tracheæ of the embryo are filled, before hatching, with a pale fluid, generally supposed to be blood-plasma. How, it is asked, can this fluid be withdrawn, and diffusion of air or of oxygen follow at once from the water in the rectum into the tracheal tubes? The obvious answer to this is, that it cannot be done. And there the matter is allowed to rest, apparently with the overthrow of the Diffusion-Theory.

Now, I propose, in this paper, to show (1), that a solution of the above difficulty *does* exist, and (2), that the Diffusion-Theory rests on solid ground, with a good morphological basis for its support.

The method of filling the tracheæ with gas in the newly-hatched larva.—It has been usually stated that, soon after hatching, isolated globules of gas appear in the tracheæ at various points, and that these, by increase and coalescence, gradually drive the fluid out of the tubes. My own observations(**7**), which support, in their chief points, those previously made by Calvert(**1**), show that this is not so. In the case of *Anax papuensis* Burm., I found that the embryo, just prior to hatching, had its tracheæ filled with liquid, as is agreed by all observers. The emergence takes place very rapidly. Owing to the interpolation of a pronymphal stage, the larva has to escape not only from the egg-shell, but also from the pronymphal sheath almost immediately afterwards. Thus there is a period of at least half a minute during which observation of the changes going on in the tracheæ is exceedingly difficult, if not quite impossible. However, at the moment the larva becomes free, *gas can be seen travelling evenly down the two dorsal trunks from the region of the midgut backwards.* It is, of course, impossible to analyse this gas; but, as we shall see later, the point is immaterial. The importance of the observation lies in the fact that the gas *does not form in the rectal region*, *i.e.*, it does not, *at the start*, pass in from the rectal cavity to the tracheæ. As the gas only travels down the dorsal tracheæ, and not down the ventral or visceral trunks, it seems almost certain that it is derived from the head or thorax, since these portions are entirely supplied by the dorsal tracheæ. We might mention, in this connection, the head-vesicle and the cephalic heart as offering a probable solution to this difficult question. Certain it is, that the pulsations of the cephalic heart synchronise closely enough with the period of the filling of the dorsal trunks with gas, to suggest that there is more than a chance-connection between the two. But this problem cannot yet be definitely solved. It is sufficient, for removing the obstacle supposed to stand in the way of an acceptance of the Diffusion-Theory, that we have shown that this obstacle does not really exist. We can take it as proved that, within a minute or two after hatching, gas fills the tracheal system of the larva, and that it comes in, not viâ the rectum, but from somewhere in the anterior end of the animal.

Now let us follow the gas-flow along the dorsal trunks. It travels steadily backwards to the rectal region. The primary efferents are first filled, then the secondary, then the smaller branches, and, finally, with considerable rapidity, gas passes up around the capillary loops, completely displacing all the liquid. The gas also passes from the dorsal tracheæ by means of short connecting branches into the ventral and visceral trunks, so that the whole system is very soon filled. The process is very easy to follow, since the gas shows up like a black rod travelling along the colourless tubes.

The expulsion of the liquid from the capillaries and larger tracheæ, which can be clearly seen taking place, is not difficult to understand if we assume (as is generally agreed) that the liquid is blood. If so, it passes into the hæmocœle, and the connection between the dorsal tracheæ and the aorta must be sought for in the cephalic heart.

As soon as the filling of the tracheal system with gas is completed, *rectal respiration* begins. The rectal valves immediately come into play vigorously, so that the rectal cavity is soon strongly distended with water. For a short time, the action is exceedingly vigorous; then it settles down to the usual slow and somewhat irregular rate.

Thus, within a few minutes of the hatching of the larva, all the conditions necessary for the passage inwards of oxygen by diffusion are fulfilled. For, whatever be the proportion of oxygen to nitrogen in the gas in the tracheal system, it is quite clear that the partial pressures of both these gases must always tend to become equal on either side of the diffusion-membrane. We may take it that the water in the rectum is fairly well aërated, *i.e.*, contains oxygen and nitrogen dissolved in the ratio of one to two.* If, then, the proportion of oxygen to nitrogen in the tracheæ be originally greater than this, it will be lowered by the passage of nitrogen inwards from the water in the rectum.

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* Or, more correctly, out of every 100 parts of gas dissolved, 35 are oxygen and 65 nitrogen. The result is obtained from the ratios of the products of the coefficient of solubility and the partial pressure of the gas at the water-surface.

If, on the other hand, (as seems more probable) the proportion be less, oxygen will pass in to a like extent. Or if, as may well be the case, the proportion is the same from the start (seeing that both egg and larva are, throughout the operation, immersed in water), equilibrium will be established from the start.

The Process of Diffusion during larval life.—Whatever be the composition of the original gas in the tracheæ, it is the oxygen in it which is required for the metabolism of the growing larva. There is, therefore, from the time that equilibrium is established, a *continuous drain* on the oxygen in the tubes. This gas is taken up at all points of the body by the cells of the developing organs, *by direct tracheal supply.* Thus there is a steady diminution of the partial pressure of oxygen going on on one side of the diffusion-membrane, while the partial pressure of the same gas on the other side of the membrane (*i.e.*, in the water) remains constant. Hence there must be a tendency for oxygen to diffuse from the water into the tracheal system *at all points where the two are brought into contact.* The problem is the same here for the integument as for gills placed in any position on the body. What we have now to see clearly is, that the suitable conditions are only set up, in our larvæ, to any appreciable extent, in the case of the rectal gills. In Fig.6, I offer a comparison between (*a*) the ordinary body-wall, (*b*) the undifferentiated rectal wall, and (*c*) the gill-wall of our larva. In (*a*), the cuticle is thick and many-layered, the hypodermis is also fairly thick, and composed of separate cells, and there is no special arrangement of tracheæ for the reception of the diffused gas (supposing that any were to pass through). In (*b*), the conditions are more favourable, in so far that the cuticle (intima) is thinner and single-layered, the epithelial cells flatter (though still separate), and the tracheal supply, on the whole, richer and finer than in the case of (i). In (*c*), finally, not only are the cuticle and epithelium reduced to the finest possible dimensions, and the cell-boundaries of the latter obliterated, but thousands of minute capillaries are actually sunk into the epithelial syncytium, so that they can receive directly the supply of diffusing gas. Could any arrangement possibly be simpler, or more perfect for the action of diffusion ?

We have already alluded to the fact that these capillaries form complete loops. This is most important, since it points strongly in favour of the view that gas must enter equally readily at all points of the circumference of the capillary. We should note, further, that each capillary passes up one wall of the gill and down the other. Thus it is arranged to the greatest advantage for the reception of oxygen from the water, which bathes both sides of the gill. Finally, as there is no spiral thread in these capillaries, and their walls are excessively fine, they offer no obstacle to the free passage of gas into their cavities by diffusion.

It is well known that *chitin*, which is the substance forming the cuticle of the gill and the intima (endotrachea) of the capillary tube, is a colloid substance which admits of the passage through it of gases by diffusion, and is particularly partial to carbonic acid gas. It is generally supposed (and the experiments of Dewitz(**2**) support this view) that chitin possesses some special power of absorbing carbonic acid gas and giving it out again. Hence we might expect that a *thick* layer of chitin would facilitate the passage of this gas, whereas, for other gases, such as oxygen or nitrogen, a *thin* layer would be necessary. This is significant when we contrast the thickness of the body-cuticle, through which carbonic acid gas is now generally believed to pass outwards, with the delicate layer covering the gills, through which we claim that oxygen passes in by diffusion.

It may be urged that the arguments applied above, in favour of the Diffusion-Theory, would be just as applicable to a theory which would substitute some chemical means of extraction in place of diffusion. May not the gill-epithelium or its pigment be an active oxygenating agent? To this the reply is that, if the gill-epithelium possessed this power, (i.) it would be sufficient for the capillaries to lie in the hæmocœle beneath the epithelium, since the oxygen would be given out to them by the inner border of the epithelium; the fact, that they are pushed in towards the cuticle, speaks in favour of diffusion; (ii.) the thinning down of the epithelium, and the fusion of its cells into a single protoplasmic mass or syncytium, would not be an advantage in this case, since a thicker layer of separate, chemically-active cells would absorb and hold far more oxygen. The question of the chemical activity of gill-pigments(4) has been seriously entertained by a number of authors, but ought to be finally disposed of by the evidence given in the case of Anisopterid larvæ, where closely allied genera may have either pure white (unpigmented), or deep purple or black (heavily pigmented) gills, and both carry on respiration equally well.

The passage of the oxygen along the tracheal tubes.—Several authors have sought for elaborate explanations of the method by which the oxygen is carried along the tracheal tubes to the various parts of the body. It was thought possible that the larger tracheæ themselves might expand or contract in cross-section, and so drive the gas forwards. This has been shown to be impossible to any appreciable extent, except in the case of vessels with specially distensible walls, such as the air-sacs of certain insects. A very curious alternative explanation has been offered by Sadones(6). He showed that, by reason of the discontinuity of the spiral threads, the tracheæ can be pulled out longitudinally, and then let back again, like a concertina. From this, he seems to have concluded that such a concertina-like action actually takes place, and is the means whereby the gas was moved along the tracheæ. This conclusion, he admits, was arrived at only after cudgelling his brains for a long time ("nous nous frappions la tête depuis longtemps")! Now it seems clear that such a supposition is not only unnecessary, but is unsupported by any morphological evidence. For the tracheæ are devoid of muscle and elastic tissue, and any extension they are capable of is purely passive, and not active. If one watches the respiration of a transparent Anisopterid larva under a low power, it will be seen that the dorsal tracheæ move up and down in the body-cavity in connection with, and not independently of, the pulsations of the rectum. There does not appear to be any change in volume, but merely a change of position. Further, if the tracheæ forced the gas onwards in any way, one would naturally expect a considerable increase in pressure to take place within the tubes, as is the case in strongly-flying imagines. But, in the Dragonfly larvæ, the large tracheal trunks do not collapse for some hours after death or dissection. This seems to be clear proof that their contained gas is at a pressure equal to, or slightly lower than, that of the atmosphere. In any case, what more is needed as a driving force than the steady consumption of oxygen by the tissues? If this consumption is rapid, surely the play of the rectal pulsations will increase correspondingly; more water will pass over the gills, and more oxygen will pass into the tracheæ from it. If the consumption is slow, the rectal pulsations will slow down also. Here indeed we note a beautiful interplay of action. For the larva uses the expulsion of water from the rectum as a means of propulsion through the water, and thus economises its store of energy by utilising the very means which supply it with oxygen!

The elimination of Nitrogen.—Though this paper is not primarily concerned with this problem, it seems advisable to point out that the question offers little difficulty, if we accept the Diffusion-Theory. For this gas will only diffuse into the capillaries in sufficient quantity to keep its partial pressure equal on both sides of the gill-wall. As the free nitrogen in the body of the larva is used little, if at all, in the process of metabolism, very little of the gas, if any, will diffuse inwards. There is, therefore, no question of the elimination of immense quantities of this gas, such as puzzle our minds in the case of the Open Tracheal System.

The elimination of Carbonic Acid Gas.—There seems to be little doubt that this is chiefly dissolved in the blood, whence it passes to the exterior through the chitin of the integument. A small amount probably gets into the tracheæ by diffusion from the hæmocœle, but the quantity would never be sufficient to affect the steady diffusion of oxygen from the gills in a supply sufficient for all requisite purposes.

In conclusion, we may say that what is now wanted, in order to make this interesting problem clearer, is a careful and accurate analysis of the gas contained in the large tracheal trunks of an Anisopterid larva (care being taken to exclude the carbonic acid gas present in the blood). Analyses made of this gas, in the case of certain insects with an Open Tracheal System, show the nitrogen to be considerably in excess of its normal proportion in atmospheric air (partial pressure of N in tracheal gas = about 90% of total pressure). Further, the total pressure within the tracheæ of such an insect exceeds the atmospheric pressure very considerably (according to Lowne, it equals 810 mm.). Thus the partial pressure of nitrogen would be 90% of 810 mm., *i.e.*, 729 mm., against 600 mm. for atmospheric air. Is it likely that similar results would be obtained for our aquatic larvæ? I think not. We might rather expect that the proportion of nitrogen to oxygen in the tracheæ of these larvæ would more nearly approach the ratio of the amounts of these gases dissolved in the water. If a ratio approaching to this latter could be obtained by analysis, it would strongly support the Diffusion-Theory. So far, I have not succeeded in devising an apparatus suitable for the necessarily very delicate analysis required, but I hope to carry this out on some future occasion.

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EXPLANATION OF PLATE XLVII.

Reference Letters.—*b*, oblique base of gill—*bp*, basal pad—*bs*, small blood-space—*cap*, capillary—*cut*, cuticle of gill—*cut′*, cuticle of body-wall, showing several, outer, coloured layers, and an inner, clear (uncoloured) layer—*ep*, epithelial syncytium of gill—*ep′*, epithelium of rectal wall—*es*, secondary efferent trachea—*hb*, hypobranchial tissue—*hy*, hypoderm—*in*, intima of rectal wall—*lam*, gill-lamellæ—*nu*$_1$, nucleus of *ep*—*nu*$_2$, nucleus of *bp*—*pg*, pigment granules—*ta*, anterior tubercle—*tp*, posterior tubercle—*tr*, large trachea.

Fig. 1.—A single gill-lamella of *Hemicordulia tau* Selys, (well-grown larva); (×95).

Fig. 3.—Three gill-lamellæ of same, viewed in profile, to show the lamellar tubercles; (×95).

Fig. 3.—Section along the line *xx* (indicated by arrows) in Fig. 1, to show the histology of the gill; (×400).

Fig. 4.—Section along the line *yy* (indicated by arrows) in Fig. 1, to show region of large tracheæ; (×400).

Fig. 5.—A small part of the distal portion of Fig. 3, much enlarged, to show histology of the gill-lamella; (×2500).

Fig. 6.—Comparison of (*a*) the body-wall, (*b*) the undifferentiated rectal wall, and (*c*) the gill-wall in dragonfly larvæ; (all three ×750).